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Full Length Research Paper

Evaluation of plant extracts on infestation of root-knot nematode on tomato (*Lycopersicon esculentum* Mill)

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Root-knot nematode, *Meloidogyne incognita*, is the major limiting factor in tomato production in many regions of the world including Ethiopia. For its management, different plant species (botanicals) are being tried in different forms as an alternative to nematicides. The nematicidal potential of baker tree (*Milletia ferruginea*), Bitter leaf (*Vernonia amygodalina*), parthenium (*Parthenium hysterophorus*), lantana (*Lantana camara*), Mexican marigold (*Tagetes minuta*), Mexican tea (*Chenopodium ambrosioides*), Neem (*Azadirachta indica*) and Pyrethrum (*Chrysanthemum cinerariafolium*) was assessed against *M. incognita* on tomato under field conditions. Aqueous extracts of the plants (20 g/100 ml w/v) were evaluated at 3 and 5% concentrations which was applied as soil drench one day after transplanting the seedling. Pronounced reduction in final nematode population density, root-knot index and a significant increase yield per plant and total yields of tomato were observed from plants treated with 5% concentration of lantana and Mexican marigold leaf extracts compared to any other tested plant extract. These plant extracts might have played an important role in reducing the nematode infestation and population buildup on tomato. Thus, these results suggest that application of lantana and Mexican marigold leaf extracts would be a good alternative to manage root-knot nematode populations in tomato production.

Keywords: Botanicals, *Meloidogyne incognita*, Root-knot nematodes, Tomato

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is an important cash crop grown by both small and commercial growers in Ethiopia. It is produced in both rainy and dry seasons. It is perhaps the most profitable crop for small-scale farmer's (Lemma *et al.,* 1992). Root-knot nematodes (RKNs), *Meloidogyne spp*., are found in all temperate and tropical areas, and are among the most damaging plant pathogens worldwide (Trudgill and Blok, 2001). In Ethiopia, Root-knot nematode species (*Meloidogyne incognita, M. javanica* and *M. ethiopica)* have been reported (Stewart and Dagnatchew, 1967). Among them *M. incognita* is the most widespread species (Wondirad and Kifle, 2000) and it is also an important problem and often a limiting factor in tomato cultivation (Wondirad and Tesfamariam, 2000).

The economic importance of Root-knot nematode on tomato is increasing as most chemical control agents for RKNs have been prohibited for environmental and health reasons. As other options, it can be managed through cultural practices like crop rotation, use of resistant varieties, biological (Abdi, 1996). However, strategies of cultural control are less effective and crop rotations are too difficult to implement because of the wide host range. In Ethiopia, varieties resistant to root-knot nematode have not been developed so far. Today there has been an increase in the intensity of search for alternative sources of effective, ecologically sound and safe control methods against nematodes.

Botanicals (plant-based pesticidal chemicals) have found favour as alternatives to pesticides in recent times. Some of these botanicals are already being exploited

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Table 1. List of Plants used against root-knot nematode in the experiment

SN	Common name	Botanical name	Plant part used
1	Baker tree	*Milletia ferruginea*	Seeds
2	Bitter leaf	*Vernonia amygodalina*	Leaves
3	Lantana	*Lantana camara*	Leaves
4	Mexican marigold	*Tagetes minuta*	Leaves
5	Mexican tea	*Chenopodium ambrosioides*	Leaves
6	Parthenium	*Parthenium hysterophorus*	Leaves
7	Neem	*Azadirachta indica*	Leaves and seeds
8	Pyrethrum	*Chrysanthemum cinerariafolium*	Flowers

commercially in insect pest management (Agnihotri *et al*., 1999). Different plant species are being tested to identify the sources of nematicidal substances and many of them have shown promising results in the control of plant parasitic nematodes outside the country (Abdi, 1996). However, in spite of the wide distribution of root-knot nematode on many crops in Ethiopia, little work has been done on the management of root-knot nematode in the country and so far, no efforts have been made to exploit locally available botanicals for the control of root-knot nematode. Therefore, this encouraged us to undertake the present investigation with the objective of investigating the effect of locally available plant extract on root-knot nematode infestation.

MATERIALS AND METHODS

Description of the study area

Plant extracts as soil drench were evaluated against root-knot nematodes *M. incognita* under field condition at Tony Farm, Dire-Dawa. Tony Farm is located at an altitude of 1197 masl and lies at 9^{o}6 'N latitude and 41^{o}8'E longitude in the eastern part of Ethiopia. The farm lies in the semi-arid belt of the eastern Rift Valley escarpment with a long-term annual average rainfall of 612 mm. The mean maximum and minimum temperature range from 28.1 to 34.6 ^oC and 14.5 to 21.6 ^0C, respectively (Belay, 2002). The soil texture of the farm is clay loam. The mean temperature during the experimental period was 27 ^oC. The average daily minimum and maximum temperature were 20 and 32 ^oC, respectively.

Field and experimental plot preparation

Seedlings of tomato, cv. Marglobe, were raised at Tony Farm nursery site for use in the experiment. The experimental plots (2.5 x 3 m) comprised rows 2.5 m long and 0.75 m wide and 0.5 m apart between hills. Each plot contained four planting rows and in each row there were five plants. The spacing between plots and blocks was 1 and 2 m, respectively. The experimental field has long history of root-knot nematode infestation but to have a uniform distribution of root-knot nematode in the field, the field was artificially infested with chopped roots of nematode infested tomato plants spread uniformly.

Plant Material Used in the Experiment

Plant materials (Table 1) were collected from mature plants of bitter leaf, parthenium, Mexican marigold and lantana from Haramaya University campus and seeds and leaves of neem were collected from Tony Farm, Dire Dawa. Mexican tea, baker tree and pyrethrum were collected from Hawassa Zuria woreda, Dilla-Wonago and Kullumsa Agricultural Research Center (KARC) Ethiopia, respectively.

Preparation of Plant Extracts

Aqueous seed, leaf or flower crude extracts of the eight plant species (Table 1) were prepared. The shade dried mature seeds, leaves and flowers were separately powdered in an electric grinder and 20 g powder of each botanical was soaked separately in 100 ml of distilled water for 24 hours in 500 ml Erlenmeyer flasks. After 24 hours of soaking, they were filtered through cheesecloth and then kept in the refrigerator until use. Each extract was considered as a standard solution "S" (100% concentration).

Determination of Root-knot Nematode Populations in Soil

To determine the initial population of root-knot nematode in the experimental field, the total experimental field was divided into three compartments. Soil samples were randomly taken from five spots in an X-manner from each compartment of the field with the help of soil auger up to 10-15 cm of soil depth and the samples were placed in polythene bags after making it composite. However, the final nematode population was determined by taking soil samples randomly from the central two planting rows and near to plant root zone from each experimental plot.

Nematodes were extracted from representative 100 ml soil taken after thoroughly mixing each soil sample. For extraction, Baermann Funnel Technique (Southy, 1970) was used. A rubber tube was fixed to the stem of a glass funnel and the tube was closed with a pinch cock to make it airtight and the funnel was then placed in a funnel stand. Water was filled in the funnel and was run out a little through the rubber tube to remove air bubbles from it. One hundred ml of soil was placed in a 250 ml beaker and covered with muslin cloth and then tied with a rubber band. The beaker containing the soil was placed upside down in the funnel containing water. To get good contact between the water and lower level of muslin, more water was added to the funnel on sides of the beaker. The assembly was kept undisturbed for 24 hours.

After 24 hrs, 10-15 ml of nematode suspension was taken into a beaker by opening the pinch cock. It was then transferred to 100 ml measuring cylinder and was made to 60 ml by adding water. It was bubbled with a pipette and 3 ml of suspension was transferred to counting dish. Population of nematodes was counted under stereoscopic microscope at magnification of 50 x and multiplied by 20 to get the population present in 100 ml of soil.

Treatments and experimental design

The experiment had a total of twenty treatment combination. Nine plant extracts were applied at 3 and 5% concentration at the rate of 30 ml/plant. There was a treated control for comparison, where fenamiphos (Nemacur 10 G) was applied @ 2 kg a.i. /ha. Untreated plots served as control too. The experiment was laid out in a randomized complete block design (RCBD) with three replications.

Treatment application

Thirty-days-old tomato seedlings (cv. Marglobe) were transplanted and one day after transplanting, the extracts were applied as soil drench around the root of the seedlings. DAP was applied at 150 kg/ha at the time of transplanting and Urea at 100 kg/ha at the time of transplanting and 30 days after transplanting in split dose. Watering and hand weeding were done as required.

Data collected

Initial nematode population density: Before treatment application, initial nematode population density per 100 cc soil was determined for the field using the Baermann Funnel Technique and was found to be 589 juveniles/100 cc soil.

Final nematode population at harvest: Final nematode population at harvest was also recorded using the method described in Section 2.5.

Root-knot index: A total of six plants from the central two rows were used for the assessment of root-knot index. The assessment was done on 1-5 scale (Taylor and Sasser, 1978). Where: 1 = no-gall, 2 = 1-25%, 3 = 26-50%, 4 =51-75%, 5 = > 75% of the root-system galled.

Yield/plant: Calculated by dividing the total fruits yield of the central two rows of the plot by the number of plants and expressed in gram.

Data analysis

The data on final nematode population were normalized using square root transformation with the formula $(x + 0.5)^{1/2}$ where x is the number of nematodes in the soil sample and 0.5 is constant number added (Gomez and Gomez, 1984). The other collected data were directly analyzed according to the standard analysis of variance (ANOVA) procedures using Statistical Analysis Software (SAS, 2002) (version.9.00, SAS. Institute Inc., Cary, NC, USA). Treatment means were separated using the least significant differences (LSD).

RESULTS AND DISCUSSION

The effect of plant extract against root-knot nematode, *M. incognita*, infestation in tomato (cv. Marglobe) was studied in the field. The extracts were applied as a soil drench one day after transplanting.

Root-knot index

The extent of galling on roots (root-knot index) is a means for detecting the infestation of *Meloidogyne* species in the roots and the damage or severity caused. In this study, all the Plant extracts were highly effective ($p \leq 0.05$) in reducing root-knot index when compared with untreated plants (Table 2). Bitter leaf, lantana, Mexican marigold, neem seed and parthenium leaves reduced galling significantly and were at par with chemical nematicide. Baker tree seeds, Mexican tea leaves, neem leaves and pyrethrum flowers also reduced galling significantly ($p \leq 0.05$) over control, but were relatively less effective.

Nematode population at harvest

The initial nematode population was 589 juveniles /100 cc soil. However, data on nematode population density in the soil recorded at the time of last harvesting (Table 2) showed that botanical extracts significantly ($p \leq 0.05$) suppressed root-knot nematode in the field over untreated control. Extracts of bitter leaf, lantana, Mexican marigold leaves and flower of pyrethrum extracts at 5% concentration significantly reduced the final nematode population over untreated control and were at par with chemical nematicide. Leaf extracts of neem and parthenium at 5% concentration were as effective as 3% concentration of Mexican marigold leaf extract. Application of fenamiphos (2 kg a.i /ha) was most effective in reducing nematode population, while 3% neem seed extract was least effective (38% reduction). In all botanical extract treated plots, the final nematode population decreased with an increase in extract concentration.

Tomato yield per plant

Application of botanicals except lower concentration of baker tree seeds and neem seeds extracts to tomato

Table 2. Effects of plant extract application on root-knot nematode infestation in tomato plants (cv. Marglobe)

Botanical	Extract Conc. (%)	Root-knot index(RKI)	Reduction in RKI over UC (%)	Final nematode population/100 cc soil	Reduction in final population over UC (%)
Baker tree seeds	3	3.3±0.30 b	20.3	463(21.5)±14 d-h	53
	5	3±0.20 b-e	26.8	455(21.3)±9.6 e-i	54
Bitter leaf leaves	3	2.9±0.20 b-f	30.1	512(22.5)±16 cde	48
	5	2.6±0.50 c-g	36.6	445(20.9)±11 f-j	55
Lantana leaves	3	2.3±0.70 fg	43.1	437(20.7)±12 g-j	56
	5	2.2± 0.08g	46.3	404(20)±10.4 ij	59
Mexican marigold leaves	3	2.6±0.90 c-g	36.6	488(22.1)± 10c-g	50
	5	2.1±0.05 g	50	401(20.1)±9.6 ij	59
Mexican tea leaves	3	3.1±0.40 bcd	23.6	512(22.6)±8.7 cd	48
	5	3±0.80 b-e	26.8	501(22)±11 c-f	49
Neem leaves	3	3.1±0.90 bcd	23.6	521(22.8)±9.4 c	47
	5	3.2±0.90 bc	22	489(22.1)±12 c-g	50
Neem seeds	3	2.7±0.09 b-g	35	610(24.7)±19 b	38
	5	2.7±0.06 b-g	35	534(23.1)±13 c	46
Parthenium leaves	3	2.5±0.25 d-g	40	605(24.6)±15.6 b	39
	5	2.4±0.70 efg	40	495(22.3)±11 c-f	50
Pyrethrum flowers	3	3.1± 0.40bcd	23.6	540(23.1)±13 c	45
	5	2.9±0.07 b-f	30.1	431(21)±10.6 hij	56
Treated control	0	2.0±0.09 g	46.3	393(19.8) ±8.9j	60
Untreated control(UC)	0	4.1±0.06 a	-	984(31.3)±19.4 a	
LSD α=0.05		0.6287		58.08 (1.30)	
CV(%)		13.6		3.51	

Treated control (Fenamiphos@ 2kg a.i /ha)
Data in parentheses are square root transformed $(x + 0.5)^{1/2}$ values
Means within the same column with a common letter are not significantly different ($p \leq 0.05$)
RKI = Root-knot index

Table 3. Effects of plant extract application on yield of tomato fruits in root-knot nematode infested field

Botanicals	Conc (%)	Tomato yield/plant (kg)	Total Yield (t/ha)	Reduction in total yield over TC* (%)
Baker tree seeds	3	1.44±0.8 gh	29.7±2.7de	43.4
	5	1.72±1.09 fg	30.9±3.1de	41.1
Bitter leaf leaves	3	1.72±0.40 fg	22.9±2.9fg	56.4
	5	2.21±1.60 e	29.4±2.6e	44.0
Lantana leaves	3	2.73±0.90 c	36.4±3.2c	30.7
	5	3.6±0.30 b	48±4.1b	8.6
Mexican marigold leaves	3	2.33±1.00de	31.4± 2.4de	40.2
	5	2.36±1.10 de	36.4±3.4c	30.7
Mexican tea leaves	3	1.87±0.60 f	24.9±2.3f	52.6
	5	2.23±1.30 de	29.7±2.1de	43.4
Neem leaves	3	1.83±0.80 f	24.4±2.1f	53.5
	5	2.24±0.60 de	29.9±2.3de	43.1
Neem seeds	3	1.45±0.80 gh	19.4±1.9gh	63.1
	5	2.25±1.20 cd	33.9±3.2cd	35.4
Parthenium leaves	3	2.22±0.70 de	19.3±1.9gh	63.2
	5	2.31±0.80 de	22.9±2.2fg	56.4
Pyrethrum flowers	3	1.65±0.70 fg	22±2.6 fg	58.1
	5	1.81±0.30 f	24.1±2f	54.1
Treated control	0	3.94± 1.20 a	52.5±3.7a	
Untreated control	0	1.24±0.60 h	16.5±2.7h	68.6
LSD α=0.05		0.3226	4.0319	-
CV (%)		8.9	8.9	-

TC* means Treated control (Fenamiphos@ 2kg a.i ha^{-1})
Means within the same column with a common letter are not significantly different (p≤ 0.05)

plants significantly (p ≤ 0.05) increased the yield of tomato per plant over untreated control (Table 3). Lantana leaf extracts at 5% and 3% were most effective and increased the yield significantly. However, the increase in yield was less than chemical nematicide. Mexican marigold leaf extracts at both level of concentration was next best in increasing the yield. The least (1.24 kg) yield was obtained from untreated control.In all botanical extracts application, yields of tomato per plant increased with the increase in concentration. In average effects of the botanicals, baker tree seed extracts were the lowest effective in increasing the yield of tomato per plants.

Total tomato yield

All botanical extracts applied on nematode infested plots significantly (p ≤ 0.05) increased the total tomato yield per hectare over untreated control plots (Table 3). Lantana leaf extract at 5% concentration resulted in the maximum total yield (48 t/ha) compared to the other botanical extracts applied. Lantana leaves with 3% concentration gave same total fruit yield (36.4 t/ha) as 5% concentration of Mexican tea leaf extract. The maximum (52.5 t/ha) total tomato fruit yield was obtained from plants treated with fenamiphos, whereas the least (16.5 t/ha) was obtained from untreated control plants.

Parthenium leaves and neem seed extracts at 3% concentration resulted in relatively lower total tomato yield than other botanical extracts applied.

Importance of organic horticultural production, which avoids synthetic pesticides applications, increased the research on botanical pesticides with potential use for nematode management (Rao *et al*., 1998). In the present field studies application of plant extract was found to reduce root-knot indices on tomato root system, final nematode population density in the soil significantly over untreated control. With the increase in level of plant extract concentration, a corresponding significant reduction was observed in the number of galls and nematode population over untreated control. Root-knot infestation stunted all untreated plants and reduced leaf production as well as tomato yield.

Lantana and Mexican marigold leaves extracts at 5% concentration resulted in a pronounced reduction in nematode numbers followed by lantana 3% concentration and pyrethrum flower extracts with5% concentration. The reproduction of *M. incognita* was significantly suppressed by all the treatments as compared to untreated plants. Similarly, all the plant extract treatments were highly effective in their ability in reducing root-knot index when compared with untreated plants. The plant extracts might be directly toxic to eggs and /or juveniles and thus reduced the root-knot nematode population density as well as galling. The decrease in number of the nematode accompanied by increase in growth of tomato suggests nematicidal potential of the tested plant extract. Similar results have been obtained by other workers too (Babatola, 1990; Akhtar and Alam, 1993; Alam *et al*., 1994).

The effects of the tested plant extracts against infestation of root-knot nematode and yield of tomato plant were different, in some cases. The differences in the toxicity of different plant extracts could be due to the differences in the chemical compositions and concentrations of toxic components. The extract might also have affected plant growth differently due to difference in chemical composition. Such results have also been reported by Firoza and Maqbool (1996) when they used different plant seeds and leaves extract against *Helicotylenchus dihystera* in tomato.

Conclusion

The present study revealed that, out of different botanicals tested, lantana and Mexican marigold leaves extract at 5 % concentration reduced root-knot nematode infestation and increased the number of fruits and total tomato yield and can be used effectively for the management of root-knot nematode in tomato and they can be used as a component of integrated root-Knot nematode managements. Also, the authors suggest that, testing the activity of Lantana and Mexican marigold with other solvents and time and method of application as a further research work.

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